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STUDIES ON THE RELATION OF THE LIVING CELLS TO TRANSPIRATION AND SAP-FLOW IN CYPERUS. I

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(WITH ONE FIGURE)

Historical discussion

Recent studies have revived with considerable vigor the doctrine that the presence of the living cells in the stem is necessary for the ascent of sap. The earlier evidence as to the necessity of living cells for sap-flow, as presented by GODLEWSKI (15) and supported by WESTERMAIER (41, 42) and JANSE (16), was shown to be inadequate by STRASBURGER (30, 31) in a series of elaborate experiments, in which long portions of stems were killed with steam, hot water, and poisons, and the dead parts proved to be still capable of conduction. Several authors have refused to accept STRASBURGER's conclusions and have presented important criticisms of his experimental work.

The vitalistic theory of sap-flow, as it may perhaps be called, has been vigorously exploited by URSPRUNG (32-37) and further supported by his student ROSHARDT (24). URSPRUNG's methods and conclusions, however, have in turn been severely criticized by JOST (17, 18), DIXON (9-12), and CZAPEK (7, 8).

URSPRUNG conceives that the living cells of the stem either may function in keeping the vessels in a proper state for conduction, or else they may take a direct part in the elevation of the sap. He believes that their lifting power is the more important function in tall plants, but that in low herbaceous ones the living cells may keep the vessels in a proper condition for conductivity. In support of his views he has carried out a series of experiments, in which portions of the petioles or stems of rooted plants were killed with heat, low temperature, or poisons.

URSPRUNG's (32) experiments were carried out on leaves of *Primula obconica*, *Pelargonium zonale*, *Begonia* sp., *Impatiens* sp.,
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and on stems of *Vicia Faba*, *Phaseolus multiflorus*, *Hedera Helix*, and *Fagus silvatica*. By treating short stretches (1, 2, or 3 cm.) of the petioles of *Primula* with steam for 3 minutes, he found that no immediate, visible effect is produced on the leaves; while if longer portions (6–9.5 cm.) are killed, wilting follows in about 4 hours. These observations are further supported by similar experiments on *Pelargonium*, *Begonia*, and *Impatiens*, from which he finds that the leaves wither more and more rapidly as the lengths of the killed portion are increased, even when the killed region is protected, and this shows, according to URSPRUNG, that wilting is not due to a lateral evaporation in the killed portion, but to a lack of sufficient water supply. He finds no stoppage of the vessels as the result of treatment. If stoppage of the vessels were caused by the treatment, he believes the leaves would wither as quickly above a short as a longer killed region.

For the study of sap-flow in stems URSPRUNG chose three plants of *Phaseolus* as nearly alike as possible. Removing all except the two topmost leaves, he killed the stems of two plants for 40 cm. above the soil, leaving 20 cm. untreated below the leaves of one plant. The killed portion was left exposed, while the other was covered with paraffin. The leaves on both plants wilted in 2–4 days, being entirely dry in 4 days. The leaves of the unprotected plants wilted faster. A plant 63 cm. high was killed for 22 cm. above the soil; the leaves remained turgescient for 19 days. From these and other experiments on *Phaseolus* he concludes that the longer the killed portion of the stem, the sooner the leaves above wither, and that the living cells are necessary to the elevation of water in sufficient quantities. On killing 80 cm. of a rooted stem of *Hedera*, the youngest leaves were observed to wilt in 1.5 days. Leaves situated below the killed region were not affected. Histological examination failed to reveal any stoppage of the vessels. In case of the stem, withering in *Fagus* could be observed in 2 days when 80 cm. of the stem were killed, while the leaves remain turgescient for 20 days when only 3 cm. were killed. In repeating some of his experiments on *Fagus*, he (34) observed that wilting and discoloration in spots occurred on the leaves. Similar discoloration was also observed when leaves were simply deprived

of water. In the case of leaves which bore spots, he observed that they still remained after drying, and concluded that they were not caused by the heating of the stem. By heating only the outer portions of a "tolerably thick" *Fagus* stem with steam for "a short time," he found that the leaves remained turgid for 9 days, and assumes that the plant can conduct water if only some of the living cells are present. In the case of several other experiments on woody stems, portions of which have been killed with steam, he found that the vessels were stopped for several centimeters above the killed portion. The wilting supervened in these cases no sooner than in other cases in which no stoppage was observed. He believes that wilting may occur in cases when stoppage is not present.

URSPRUNG (34-37), parallel with steam, has also employed girdling experiments on about 20 species of plants besides *Fagus*. When 10 cm. of richly leaved branches of *Ulmus* and *Populus* are killed with steam, the leaves wilt in 1-2 days, while in *Prunus* they last for 25 days under similar treatment. He has also killed branches and stems for a length of 3-10 cm. near the top and near the base. The nearer the killed stretch is to the leaves, the sooner they wither. With the exception of *Prunus*, the leaves remain longer turgescient, some ten times as long, when 3 cm. than when 80 cm. are killed. His girdling experiments gave very divergent results in the different plants studied. *Viburnum* kept its leaves turgescient for 45 days; *Ulmus* leaves began to wilt in 1 day. URSPRUNG concludes from these heating and girdling experiments that two factors are concerned in the death of the leaves: (1) increased resistance to sap-flow, (2) diminution of the power of transportation. He believes that the very great difference in time of wilting in different plants is due not to differences in their sensitiveness, but to a difference in their capacity to conduct and hold water under the condition of the experiment. A slow water transport may be due to an increased resistance to flow or to a decrease in the power of the transportation of the stem. Since stoppage of the vessels is not universally present in treated stems, he thinks that the increased resistance to flow is not to be considered. According to him the transport of water is partly

physical and partly vital, and the vital factor is eliminated by killing a portion of the stem.

URSPRUNG (34) has also used ether, induction currents, and low temperature to kill portions of the stems of both cut and rooted plants. By removing half of the cortex for 10 cm. from the branches of *Fagus* and exposing the wound to ether, the leaves were observed to wither in 2-3 days. By cooling portions of the branches of the same species with ice for 12-30 cm., the leaves above were always observed to wilt in 2-6 days, depending on the length of the cooled portion. All of these researches cause URSPRUNG to conclude that the living cells are in some way essential to the ascent of water.

STRASBURGER also performed some experiments on plants in connection with the roots, killing various stretches of the stem with heat. URSPRUNG (32, 33) in discussing these experiments notes that the leaves above fade, and concludes that the water does not pass through the killed portion in sufficient quantities to supply the leaves. He thinks, therefore, that STRASBURGER'S experiments show that the living cells of the stem are necessary in order that a sufficient amount of water may ascend to supply the leaves. He further cites in favor of his own view the experiments of BOEHM (3), in which 18 cm. of stems of bean plants were killed with steam, noting that the leaves above "lived" for a period not longer than 3 weeks; wilting usually occurred sooner. BOEHM considered that the wilting was due to a plugging of the vessels with mucilage or to a severing of the water columns. URSPRUNG passes over this observation with the remark that it teaches nothing new.

Considering the possibility of the participation of the living cells of the stem of a plant in sap-flow, DIXON (9) has repeated some of the experiments of URSPRUNG (32) with rooted stems and attached leaves. Although he fully supports URSPRUNG'S account, he gives quite a different interpretation of the results obtained. When only "short lengths" of the stems of *Primula*, *Chrysanthemum*, *Syringa*, *Philadelphus*, and *Cytisus* were killed by heat, the leaves beyond "scarcely suffered," little injury appearing; but when 2-5 cm. lengths were killed, the leaves showed injury in proportion as the length of the killed region was increased.

This progressive effect DIXON attributes to the probable introduction into the leaves of poisonous or plasmolyzing substances from the dead cells. Three branches of *Syringa* were placed in water and three others in a filtered decoction made by boiling stems of the same kind and filtering and cooling the fluid. The stems which stood in the decoction wilted its leaves in 2 days, while the stems in fresh water wilted their leaves in 5 days. As compared with stems set in fresh water, wilting took place much faster in those placed in the decoction. In order to show that this effect was not due to a clogging of the vessels, the immersed ends of the stems were frequently cut away. The injurious properties of the decoction remained, showing that wilting was probably not due to a clogging of the vessels by comparatively impermeable substances, since these would have been removed by the repeated filterings. DIXON suggests that it is possible that the application of heat in these experiments may to some degree have permanently interrupted the water supply by breaking the water columns.

URSPRUNG (35-37) has repeated DIXON's experiments described above, using *Impatiens sultani*, and has reached the same results. Microscopical examination showed him that the vessels at the base of the stem were plugged with a brown mass. Upon bringing a plant which had stood in a decoction into a moist room, the leaves again became turgescerit. When stems which had become stopped by standing in a decoction were placed in water, the leaves rapidly became turgid. The leaves of plants which had been standing in a solution of CuCl_2 remained wilted when brought into a moist room. He was unable to find stoppage in plants which had been placed in CuCl_2 . He concludes, therefore, that his researches show that the wilting of the leaves is not due to a poisonous action of the decoction, but to the insufficient water supply caused by the stoppage of the vessels. He further placed a rooted *Impatiens* plant in a concentrated *Impatiens* decoction. After 2 days, microscopical examination showed that the protoplasm of the root hairs was in a perfectly normal condition. The leaves of this plant remained completely turgescerit, while those of a cut shoot placed in a decoction rapidly wilted. The decoction possesses, therefore, according to him, no plasmolyzing substances.

DIXON (10-12) finds that water forced through a steamed portion of a stem is tinged with a brown substance, while water forced through an unsteamed or otherwise heated stem emerges as a clear liquid. He also performed experiments on cut branches of *Syringa*, placing some in water and others in a decoction of the same plant. The leaves on the stems set in water wilted in 5 days; those set in the decoction wilted in 3 days. URSPRUNG, performing similar experiments with *Impatiens* and obtaining similar results, does not believe that the leaves fade because they are poisoned by the decoction, but that they fade because of a stoppage of the vessels at the cut end of the branch with a brown mass which interferes with the water supply. Among other experiments along this line, DIXON killed one arm of a bifurcated *Syringa* shoot with hot water. The leaves were then removed and pure water was supplied through it to the living branch, so that the leaves on it received their water supply through the main stem and through the dead branch. In spite of the ability of the leaves on the living branch to receive their water supply from the roots as well as through the dead branch, they soon showed signs of wilting, resembling the early stages of fading characteristic of the leaves above a heated portion. DIXON thinks that the leaves fade because substances are formed during the heating of the stem which are carried to the leaves and act injuriously upon them. He further concludes from his experimental evidence that the fading of the leaves on a heated stem is not the same as occurs normally when leaves are simply deprived of water. In leaves above a killed stretch he observed a contraction of the protoplasts of the mesophyll cells and a discoloration of the chloroplasts in the areas which were turning color. DIXON holds, therefore, with VESQUE (38, 39) that in one case the leaves die because they dry, while in the other case they dry because they die, which appears from my observations to be a true statement of the facts.

ROSHARDT (24), proposing to study the problem of sap-flow as outlined by URSPRUNG, has performed experiments on 131 species of 59 families of shrubs and herbaceous seed plants, by killing certain stretches of the petioles, stems, and branches with steam, ether, xylol, or low temperature, and determining what effect the

treatment may have on sap-flow. For comparison with my observations on *Cyperus*, ROSHARDT's experiments on the grasses are of particular interest. Experiments were made upon *Bromus sterilis*, *Bromus hordeaceus*, *Dactylis glomerata*, *Poa pratensis*, *Agropyrum repens*, *Glyceria plicata*, *Arrhenatherum elatius*, *Lolium perenne*, *Cynosurus cristatus*, *Secale cereale*, *Miscanthus polydactylos*, and *Bambusa aurea*. With the exception of *Secale*, *Miscanthus*, and *Bambusa*, 5-10 similar halms were killed for the same stretch. Although the grasses agree generally in the morphological structure, he finds that their response to the treatment was widely different. He was unable to observe distinct wilting of the leaves except in *Bromus hordeaceus*, and then only in direct sunlight. In other species of the grasses, yellow-red spotting together with a yellow discoloration of the leaves appeared, which was followed by shriveling. The leaves began to dry first at the tips and fading began from below upward, the upper leaves drying last. The flowers and fruits remained fresh longer than the leaves, and the halms dried last. The leaves of *Miscanthus* wilted from above downward on the stem. The lack of water was often indicated by a rolling of the leaves, which occurred in *Agropyrum* and *Bambusa*. He tabulates the results in cases in which a length of 10 cm. of the halms was killed in order of their drying: *Bromus sterilis* (dry in 17 days), *B. hordeaceus* (16 days), *Bambusa* (13 days), *Dactylis* (12-14 days), *Glyceria* (10 days), *Poa* (9 days), *Lolium* (9 days), *Arrhenatherum* (about 8 days), *Miscanthus* (7 days), and *Secale* (about 7 days). When 20 cm. of the halms are killed, the following order results: *Agropyrum* (12-13 days), *Bromus hordeaceus* (12 days), *Bambusa* (10 days), *Glyceria* (8 days), *Poa* (5-7 days), and *Miscanthus* (2-5 days when 22 cm. were killed). It will be seen that the leaves of *Bromus hordeaceus* remain fresh 16 days when 10 cm. are killed, while its leaves dry more quickly (12 days) when 20 cm. are killed. He feels justified in concluding from these results that the longer the killed stretch the sooner the leaves above indicate a deficiency in the water supply by drying.

ROSHARDT's tables show that killing the same stretch of the stem of nearly related forms of the same genus produces very different effects in the time of wilting of the leaves. He has compared

several plants as to time of wilting when 10 cm. of the stem were treated, with the following results: *Hypericum* 17 cm. (22 days), *Lycium* 20 cm. (16-17 days), *Physalis* 20 cm. (9 days), *Anthriscus* 16-20 cm. (about 10 days), *Pisum* 27 cm. (9 days), *Hemerocallis* 20 cm. (9 days), *Sambucus* 20 cm. (about 9 days), *Rheum* 12 cm. (12 days), *Bromus sterilis* 10 cm. (12 days), *Spiraea Thunbergii* 13 cm. (11 days), *Ampelopsis* 8 cm. (12-13 days). The leaves of the following plants withered very quickly when 10 cm. of the stems were killed: *Polygonum virginianum* 2.5 cm. (0 days), *Circaea lutea* 2.5-3 cm. (0 days), *Ficaria* 9.5 cm. (0 days), *Primula obconica* 10 cm. (0 days), *Malva* (petiole) 10 cm. (0.5 day), *Urtica* (petiole) 2 cm. (about 1 day), *Campanula rapunculoides* 2 cm. (1 day), *Petunia* 2 cm. (1 day), *Crepis* (young plant) 5 cm. (1 day), *Narcissus Pseudo-Narcissus* 6 cm. (1 day), *Gentiana lutea* 9 cm. (1 day), *Adenostyles* 10 cm. (1 day), *Verbena* 13 cm. (1 day), *Cannabis* 8 cm. (about 1 day), *Helianthus* (young leaves) 3.5-6 cm. (1-2 days), *Primula elatior* 2-7 cm. (1-1.5 days).

Besides using steam and low temperature to kill various stretches of the stems and petioles, ROSHARDT has employed ether and xylol, applying the fluid to the parts with a fine brush. The petioles of 8 plants of *Arum maculatum* were treated with ether, and those of 12 other plants with xylol for a distance of 7-14 cm. He found that the leaves wilt, discolor, and dry in 9-14 days. By treating a 23 cm. stem of *Phaseolus multiflorus* with xylol for a 10 cm. stretch, the leaves were observed to wilt in 2 days and to be dry in 4 days. The leaves of *Tropaeolum*, whose petioles were painted with xylol for 6-20 cm., wilted in 2-4 days and dried in 4-7 days; controls cut and placed in the neighborhood wilted in 2 days and dried in 7 days. He carried out experiments on *Oxalis Acetosella*, observing what effect the painting of the petioles with ether or xylol would have on the sleep movements of the leaves. A number of petioles of 12 plants were painted with ether for "short stretches." Immediately the leaves assumed the characteristic sleep positions, which they kept until evening; they had assumed their normal positions by the next morning; after 4 days, discoloration and wilting took place. A control plant was stroked with a brush without the ether and the leaves behaved normally. Por-

tions of the petioles of 4 plants of *Oxalis* were painted with xylol for 3 cm.; the plants failed to assume the sleep position; the leaves wilted in 2 days and dried in 3 days. Control plants wilted in a few hours after being cut. ROSHARDT is inclined to think from these results that ether is absorbed and carried to the leaves, while the xylol is not carried to the leaves. He has performed other experiments on other species with these two substances with similar results.

In order to test further the truth of this hypothesis, ROSHARDT has performed experiments somewhat similar to those which I shall describe, the results of which appear in tables V, VI, VIII, and IX. He has interpreted his results, however, in an entirely different manner. Rooted plants of *Arum maculatum*, *Phaseolus multiflorus*, *Malva neglecta*, *Convallaria majalis*, and *Physalis alkekengi* were experimented upon; two similar plants of each species were placed in a PFEFFER'S transpiration apparatus. One plant was left undisturbed and a section of the stem was steamed for a certain time. The water absorbed was calculated in volumes, and the amount transpired was determined by weighing at regular half-hour intervals. The investigation was carried on until wilting began. In his table (p. 352) which represents his results on *Arum*, it will be noticed that the amount of water transpired during the first half-hour after treatment is 0.095 gm., while 0.110 gm. was given off during the second half-hour. During the 3rd, 4th, and 5th intervals, 0.090, 0.095, and 0.100 gm. respectively are lost. The succeeding periods also show some increases over the amount of water transpired during the preceding ones. It is noticeable that in the 6th interval no water whatever is given off. From these quantitative results ROSHARDT concludes that apparently neither poisons, nor displacement of the contents of the vessels, nor a stoppage of the tubes occur as a result of killing the stem with heat, but the final (though not continuous) decrease in the water transport is due to a lack of energy, which the dead cells are unable to supply.

EWART (14) adheres to the vitalistic theory, and, after repeating STRASBURGER'S experiment on a maple tree 15 m. high, concludes that picric acid is not a satisfactory poison for killing the stem

cells in such an experiment, owing to its retention in the walls of the wood vessels and its inability to diffuse well laterally. In an experiment in which the base of the trunk was first placed for 2 days in formic aldehyde, after which eosin was added to the solution, the latter rose to the top in 4 days. EWART finds, however, that in this case eosin rises not in the poisoned part of the wood, that is, the outer younger rings, but in parts which the formalin had not penetrated at all or only in very dilute form and which were still alive. Even in this case the flow of eosin solution was only temporary, nearly all conductivity being practically lost in 5 days. EWART concludes that his results apparently show that eosin solution following formalin instead of rising in the young poisoned portion of the wood passed upward through the unkilld parts. "The ascent of sap in trees must be regarded as a vital problem in which vital actions, directly or indirectly, take part," and is "a vital problem in so far as it depends upon conditions which hitherto can only be maintained in living wood."

REINDERS (23) found that manometers placed one above the other on the same stem behave quite independently of one another, sometimes one and sometimes another showing the lower pressure. In a suspended water column the pressure decreases gradually toward the top. On account of the differences of pressure observed in *Sorbus latifolia*, *Cornus*, and *Syringa*, REINDERS feels constrained to conclude that the living wood is normally able to pump water actively. The irregularities in his pressure measurements led him to seek for the cause in the activity of the living cells of the wood. He believes that the irregularities would disappear if these influences were removed; therefore he killed with steam a 2 m. stem of *Cornus* in two places for a distance of 10-12 cm., the distance between the killed portions being 66 cm. He also killed a 2.5 m. branch of *Sorbus* throughout its length, observing that the manometers at once showed more regularity; the leaves on this stem remained uninjured for 3 weeks. Four manometers were attached to the stem of a 2 m. *Syringa vulgaris* bush; the instruments after a short period all showed an equal "suction," which soon varied during the night and day, although the differences in pressure were small, a higher pressure sometimes being indicated in one and

sometimes in another. After 15 days a branch bearing one of the manometers was killed, together with a portion of the stem to which it was attached, by passing an induction current through them. After at first showing slight variations in pressure, this manometer followed the same periods as the others, but always "sucked" slightly more. REINDERS has given us only a preliminary account of his experiments, and the final report will be awaited with interest.

ZIJLSTRA (43), after having kept 50 cm. of the stems of a small apple tree and several plants of *Polygonum cuspidatum* and *Helianthus tuberosus* cooled to 0° C. 6-8 days, finds no fading of the leaves during that time, while leaves on cut branches hung in the neighborhood rapidly withered. In spite of this seemingly convincing result, he still believes that the living cells are necessary to sap-flow, as held by his associate REINDERS. His explanation is that it is quite probable that 50 cm. is too short a stretch to show conclusive results, although he did not take the trouble to treat longer portions. Both of these investigators, therefore, advocate the doctrine of a pumping action of the wood, as formulated by GODLEWSKI, for which the living cells are necessary. ZIJLSTRA allowed a solution of "säure violett" to ascend dead and living branches, and then examined them microscopically. In the living ones only the tori of the bordered pits were stained, together with a thin layer of the walls of the vessels. In the dead branches the whole of the wood was uniformly colored. He concludes, therefore, that the ascending water takes quite a different course in dead wood from that in living wood.

JOSEPH (17, 18) points out that URSPRUNG's method of experimentation is an indirect one, and that, if it is not due to an increased evaporation in the scalded portion or to the stoppage of the vessels, which URSPRUNG was unable to find, then it must be due to the death of the parenchyma cells. He suggests that by heating the stem perhaps changes occur in the walls or in the contents of the vessels which one cannot readily observe with the microscope.

CZAPEK (7, 8) also doubts the worth of such experiments as described by URSPRUNG, since in the killed portion physical disturbances may be induced, such as drying out of the cells walls or

stoppage of the vessels, which in themselves would influence the passage of water even if the killed cells were otherwise capable of conduction. Both JOST and CZAPEK doubt whether all changes induced in the cells by heat can be seen with the microscope. The experiments of WEBER (40) on heated portions of *Picea excelsa*, in which he found that both boundaries of the treated region between the dead and living portions were unable to transmit water even under pressure, were confirmed and extended by JANSE (16). WEBER further detected a transparent substance in the vessels. The experiments tend to show that a stoppage of the vessels may occur in heated stems, and also that a partial poisoning of the leaves follows the death of the cells of the stem, since withering of the leaves begins before stoppage of the flow can be observed, as has been shown by DIXON.

In microscopically investigating the leaves of *Populus*, *Tilia*, *Syringa*, *Salix*, and *Acer* borne above a treated stretch, DIXON (10) found that the mesophyll and the walls of the tracheae of the leaves become discolored before wilting occurs, which led him to suspect that the leaves are dying from other causes than mere drying. ROSHARDT contends, however, that the discoloration of the mesophyll and veins is no more an indication of poisoning than it is of mere lack of water, and that the discoloration of leaves on a stem with a killed section is not nearly so general as DIXON implies. As further proof that injurious substances are caused by heating the stem, DIXON mentions instances of leaves wilting directly below the killed stretch. ROSHARDT states that in all of the 800 species which he treated he could not find wilting in any of the leaves below the killed portion. That substances can be forced downward by steaming the stem is shown by my observations on *Cyperus*. I have found that some vessels below the killed portions were often totally or partially filled with resinous substances, even as far as the roots. ROSHARDT, on the other hand, failed to observe such stoppages either above or below the heated region. As further evidence that substances may be forced downward, my observations on *Cyperus* show that when cut stems are placed in water and the stem heated, numerous air bubbles are forced out of the cut end by the treatment.

URSPRUNG (35-37) holds that DIXON's idea that poisonous substances are carried to the leaves from the heated portion cannot be the correct one, since he finds that poisons such as CuCl_2 cause fading in quite a different way from that which occurs in the case of leaves on heated stems, and very much more quickly than is caused by a decoction of the stem supplied to the leaves in the manner described by DIXON. I can see no reason whatever for supposing that a metallic poison should have the same effect as the toxins which may be engendered by heating the stem, nor that the time required should be the same in both cases. By placing the faded leaves of an *Impatiens* branch, which had stood in a decoction, in a moist atmosphere, URSPRUNG observed that they soon regained their turgescence. He thinks this shows that the leaves are not affected by plasmolyzing substances of a decoction of the plant. It may be, however, that these substances exert their plasmolyzing influences more slowly, and that the turgescence may be recovered if the leaves are not dead. It is well known that leaves may undergo a very marked degree of wilting and still recover their turgescence. SCHROEDER has shown that most leaves can lose as much as one-half of their water content without severely suffering.

SCHROEDER (27), in studying the symptoms of death as a result of wilting, finds that in most leaves a discoloration occurs, due to an oxidation of the tannin content. He also observed that in the early stages of death the chloroplasts move to one end of the cell or toward the middle; they round up and lose their typical color and structure. The protoplasts finally contract, the plastids take on a glassy appearance, and Brownian movements are observable in the cell contents. In the microscopical studies which I have made of the leaves on steamed stems, I have found many of the same conditions described by SCHROEDER, all of which indicates that these leaves are dying, not so much, it seems to me, from a lack of water as from the effect of some harmful substances. He finds that the rate of loss of water decreases from the beginning to the end of the process. The amount given off during any one interval is never greater than that of the preceding one. Death of the leaves greatly modifies the successively decreasing rate of water loss during wilting. The first 50 per cent of the fresh weight

of the leaf is usually very rapidly lost when dead, after which the amount decreases more uniformly.

JOST puts the question clearly in maintaining that "a completely convincing experiment must show that the leaves which are supplied with water by a dead stem are able to remain for a long time alive." The difficulty so far has been, in part at least, to render the living cells of a stem inactive without causing other changes, or only such as may be ignored. It has been observed by BOEHM (3) and STRASBURGER (30), among others, that in experiments in which certain portions of a stem are killed with steam, the leaves above these regions wither and die sooner than in the control, a phenomenon which KOSAROFF (20, 21) also observed in his experiments on cooled stems. That water passes through the scalded or cooled region has been shown many times by placing cut ends of the stems in dyes. That it does not pass in sufficient quantities may perhaps be the cause of the earlier withering of the leaves on a branch, a section of which has been killed, as has been urged by URSPRUNG, EWART, and by ROSHARDT in support of the vitalistic theory of sap-flow. URSPRUNG employed steam and low temperature to kill sections of the stem in certain woody plants, or removed the bark without otherwise disturbing the connection between root and stem. In addition to steam, ROSHARDT has used low temperature, and in certain cases xylol or ether. According to these authors, earlier withering of the leaves is a sure sign that a sufficient water supply does not pass through the killed portion. Both authors have shown that the longer the killed portion is, the more quickly the leaves above wither and fade, as was formerly observed by JANSE (16).

That SCHWENDENER (29) still adheres to the vitalistic hypothesis of sap-flow is shown by certain passages in the last edition of his lectures on mechanical problems in botany, in which he says:

Ohne dieses Eingreifen (Lebensthätigkeit der Zellen) ist die Hebung des Wassers auf Höhen von 150–200 Fuss und darüber einfach unmöglich und alle Bemühungen, die vorhandenen Schranken mit unklaren physischen Annahmen zu durchbrechen, sind nicht viel mehr als ein Suchen nach dem Stein der Weisen.

LECLERC DU SABLON (22) believes that the ascent of sap is easily explained by the osmotic qualities of the living cells of the

leaves, stems, and roots. The transpiration of the leaves and the absorption by the roots sets up a difference in pressure at the two ends of the plant. The principal factor, however, in the ascent of sap is the osmotic power of the wood parenchyma, which tends to keep the quantity of water constant. He thinks that transpiration simply serves to accelerate the rate of flow, but does not cause it ("Le mécanisme des mouvements de la sève est donc le même dans un arbre haut de 100 mètres que dans une herbe de quelques centimètres, dans une tige verticale que dans une tige horizontale").

DIXON's theory of sap-flow is perhaps the most thoroughly in accord with the experimental facts as they stand today. He holds that the elevation of water even in the highest trees is due to a tensile stress being set up in the tracheae of the leaves when water is abstracted from them by the evaporation from the leaves, which stress is transmitted to the water in the larger vessels. The water adheres to the walls of the vessels, so that the stress is resisted. The water "hangs" in the vessels "by virtue of its cohesion." This theory has been objected to on the ground that the water columns are not continuous, and that air bubbles and vapor are present in the vessels. DIXON points out that owing to the permeability of the walls of the vessels, the water does form a continuous system. It will be seen that this theory eliminates the action of the living cells of the stem. DIXON cites the classic experiments of STRASBURGER (31) in which an oak 22 m. high was sawed through and placed in picric acid for three days until the liquid had risen 3 m. The tree was then placed in fuchsin, which rose 18 m. in the dead stem in 8 days. DIXON believes that this experiment proves conclusively the uselessness of the living cells of the stem in sap-flow.

Preliminary experiments

It is plain that the question as to the effect of killing a section of a stem on the character and quantity of the sap-flow is still unsettled as to many particulars. There is no question that, as URSPRUNG admits, such stems conduct a certain amount of water for a considerable period of time. Experiments conducted on a quantitative basis are essential in such a problem, and the following studies have been carried on from this standpoint. After

several years of experience with classroom experiments, it has been found that the common umbrella plant, *Cyperus alternifolius*, is a very suitable species for such experimentation. The plants are easily cultivated in the greenhouse, often reaching 60-90 cm. or more in height. The aerial stems are free from nodes and leaves except at the top, where the large, many-rayed involucre of narrow leaves is situated. The younger leaves are comparatively translucent, permitting easy observation of the rise of colored liquids such as eosin solutions. As will be seen below, the plants transpire rather rapidly as compared with those in ordinary use for laboratory experiments, giving off fairly large quantities of water per unit area of leaf surface. The rate of sap-flow is of course correspondingly rapid. Further, the stems and leaves are not easily injured by the mechanical manipulation often necessary in such experiments. *Cyperus papyrus* is also an excellent plant for comparative study on account of its longer stems, but the fading of the leaves is less readily observed, owing to their narrow threadlike form.

The character of the involucre leaves allows a glass tube or LIEBIG condenser of suitable diameter and length to be readily slipped over the crown and down on the stem without injury to any part of the plant. Two-holed rubber corks may be split and clamped about the stem above and below and fitted into the glass tube. If small glass tubes are bent at right angles and inserted into the remaining holes of the corks, an inlet and outlet for steam is easily arranged. The steam may be conducted away to any distance so as not to injure the plants except in the desired region. The method of arranging the experiment is shown in fig. 1. By clamping the rubber connections and leaving the incasing glass tube on the plant after treatment, the stem is inclosed in a sterile chamber and is kept in a moist atmosphere which prevents its drying and does away with the necessity of using paraffin or other protective covering. If it be desired to use melted paraffin or wax, this may be poured into the tube, thus inclosing the injured region. In using a poison instead of steam, the fluid may be poured into the tube at the top and drawn off at the bottom after the treatment is finished. I have successfully used grafting wax to further secure the corks and connections and prevent

poisonous solutions from leaking out and injuring the stems below the chamber. Such an arrangement has the added advantage of allowing the treated portion of the stem to be easily observed

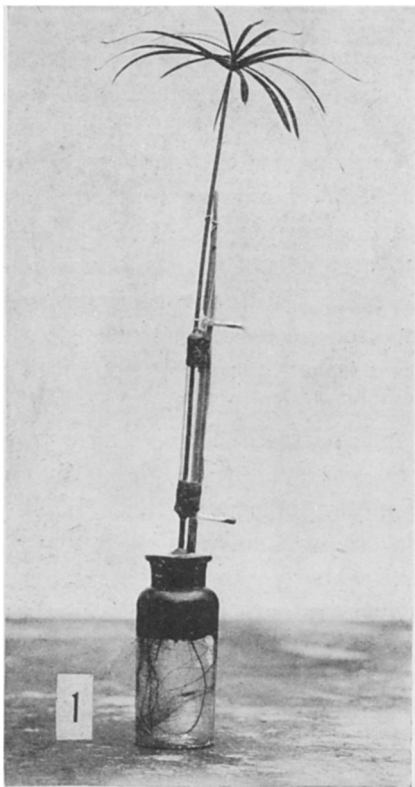


FIG. 1.—Photograph of *Cyperus* plant having all but one leaf removed and growing in a nutrient solution; this photograph shows the incasing glass tube and connections arranged for steaming a portion of the stem.

during the experiment. For killing sections of the stem I have used steam, hot wax, and several poisons, treating sections of the stems for any desired length. As poisons I have employed saturated aqueous solutions of picric acid, mercuric bichlorid, copper sulfate, potassium hydroxid, 1 per cent aqueous solution of chromic acid, 4-40 per cent formalin, alcohol, xylol, chloroform, and ZENKER'S fixing fluid. In every instance I have killed both shorter and longer stretches of the stems, and have compared the time required for the withering of the leaves, and the amount of water absorbed and given off, with the same data from an untreated control plant cut and standing with its base in water. I have also made some microscopic examinations of the stems above and below the killed portions, especially in

the zone between the killed and living region, in order to determine if possible whether any visible plugging or stoppage of the vessels or other changes above the killed zone occur. Microscopic examinations were also made to determine to what extent poisons penetrated the cells of the stems and the leaves as indicated by

their plasmolysis. I have further experimented in the same way with cut stems, in order to compare the amount and rate of transpiration with that of stems treated in the same way but kept in connection with the roots. Since several recent workers have raised objections to the use of cut plants in such experiments, this point is worthy of careful consideration. Throughout the investigation, all plants were kept as nearly as possible under similar conditions of light, temperature, and moisture.

To determine the amount of water lost per unit area of leaf surface by the umbrella plant as compared with other common forms such as *Helianthus annuus*, potted plants and plants grown in culture solutions were used. In such experiments all of the stems but one were cut away, and the soil and pot were then so inclosed as to allow no loss of water except through the leaves. In case of plants grown in culture solutions the corks were sealed in. For these cultures 500 c.c. wide-mouth bottles were used. Flat corks to fit were split and fitted about the stems of the plants after they had first been wrapped with cotton wool for protection. The corks were sunk about 1 cm. below the edge of the bottle, so that melted wax could be poured in when it was desired to seal in the corks. The plants were grown in KNOP'S nutrient solution as given by DETMER. Leaf areas were determined by using EASTMAN'S ferro-prussiate paper; the white areas were cut out and weighed. By obtaining the weight of 1 sq. cm. of the blue print paper, the areas of the leaves were easily obtained. In order to determine whether the paper was uniform in weight, several weighings were made. The average weight of 1 sq. cm. was used as the unit in calculating the leaf areas. This of course must be doubled in determining the transpiring area if stomata are on both sides of the leaves, which is not the case in the normal plant. In order to calculate the rate of motion of the transpiration stream, the lithium method as described by SACHS (25) for rooted plants, as well as the eosin method, was used. In the latter case the rise of the eosin in the stems and leaves of cut branches was observed, as is easily possible in the umbrella plant by the aid of the horizontal microscope.

Some of the experiments described by URSPRUNG and ROSHARDT

have been repeated, with similar results. Some experiments have also been made on plants other than *Cyperus*, but in this paper my description will be confined to this plant.

SAMPSON and ALLEN (26), in studying the influence of external factors on transpiration, have found that "variation in the transpiration per unit area for a given time is found to be slight for plants of the same species, when about the same age, and grown and tested under similar conditions"; facts which my observations on *Cyperus* fully substantiate.

The following table (I) shows the variation in the total daily amount of water lost in *Cyperus* plants of nearly the same age and size when grown and tested under our greenhouse conditions. The 5 plants in each series were selected with reference to likeness, and the weighings were made at the same time for 10 days, the average of which was taken as the average daily transpiration loss. The average daily temperature was calculated from the daily record of a self-registering RICHARD thermograph placed in the neighborhood of the plants. This is necessary, since the changes in temperature affect the transpiration rate to a very marked degree. The plants in series I were young, with their involucreal leaves just expanded; those in series II were well developed, dark-green plants.

TABLE I
SHOWING VARIATION IN AMOUNT OF WATER LOSS BY PLANTS OF APPROXIMATELY THE SAME AGE AND SIZE

SERIES I			SERIES II		
No. of exp.	Average daily water loss in grams	Average daily temperature	No. of exp.	Average daily water loss in grams	Average daily temperature
1.....	4.5	24° C.	1	1.8	20° C.
2.....	4.7	24° C.	2	1.9	20° C.
3.....	5.0	24° C.	3	1.6	20° C.
4.....	4.6	24° C.	4	2.0	20° C.
5.....	4.2	24° C.	5	1.7	20° C.

I have determined by the pot method and by means of plants grown in nutrient solutions that the average amount of water transpired per unit area for a given time is 4-10 mg. per sq. cm. per hour during a period of 24 hours. In comparison with the earlier

observations of SACHS on the amount of transpiration in *Helianthus* (about 2.23 mg. per sq. cm. per hour) and with those of more recent investigations, it is evident that *Cyperus* may at least be regarded as a rapidly transpiring plant under greenhouse conditions. Miss CLAPP (6), in comparing a number of common plants as to transpiration, found that *Helianthus annuus* stands far ahead of the 30 other forms which she investigated. A plant with a total leaf area of 5.056 sq. cm. gave off on the average 25.7 mg. of water for each sq. cm. of leaf surface per hour under ordinary greenhouse conditions. Hence *Cyperus* gives off a much smaller amount of water per unit area of leaf surface per hour than does *Helianthus*.

The leaf stems have numerous stomata over their surfaces, while these structures are confined almost entirely to the under surfaces of the leaves. By removing the involucre of plants grown in nutrient solutions and sealing the cut ends of the stems with grafting wax, then making weighings, the daily transpiration per unit area of stem surface was found to be on the average about 3.5 mg. per sq. cm. per hour. After having measured the average daily water loss of a plant in culture solution through its leaves and stems, the involucre was removed, the cut ends sealed, and weighings resumed for several successive days.

TABLE II

SHOWING AMOUNT OF DAILY WATER LOSS BY A PLANT BEFORE AND AFTER
REMOVING THE LEAVES

PLANT WITH LEAVES			PLANT WITH LEAVES REMOVED		
Days	Daily loss in grams	Average temperature	Days	Daily loss in grams	Average temperature
1.....	17.4	23° C.	1	2.2	25° C.
2.....	19.9	25° C.	2	2.1	23° C.
3.....	18.1	24° C.	3	2.1	24° C.
4.....	18.6	24° C.	4	2.2	25° C.
5.....	19.4	25° C.	5	2.2	25° C.
Average daily transp. loss 18.68 grams			Average daily transp. loss 2.18 grams		

The data tabulated in table II were obtained by using a large, well rooted plant growing in a nutrient solution. The stem was 35 cm. long, 4 cm. in diameter, bearing an involucre of 17 large rays.

The temperature was obtained by averaging the daily record as registered by a RICHARD thermograph. It is seen from the table that a plant giving off 18.6 gm. of water per day through its leaves and stem will transpire 2.2 gm. only through its stem.

Observations with lithium nitrate show that water rises in stems of plants in nutrient solutions whose tops have been removed, at the rate of about 150 mm. per hour. SACHS (25) concluded that the transpiration current travels at the rate of 63 cm. per hour in *Helianthus annuus*, and that the rate of sap-flow varies greatly in different plants and in the same plant under different conditions. The method of measuring the rate of sap-flow by setting cut stems in dyes and observing the rise of the colored solution has been criticized by SACHS, who showed that the water travels faster than the dye. A more exact method used by him consists in watering the entire plant with a solution of some lithium salt, or adding the salt to nutrient solutions, and ascertaining by means of the spectro-scope how far the salt rises in a given time, thus obtaining the rate of flow.

TABLE III
SHOWING THE RATE OF SAP-FLOW IN ROOTED PLANTS AS DETERMINED BY
SACHS'S LITHIUM METHOD

Length of plant	Time taken to ascend	Rate per minute	Rate per hour
73 cm.	24 min.	3.04 cm.	182.4 cm.
50 cm.	20 min.	2.50 cm.	180.0 cm.
45 cm.	15 min.	3.00 cm.	180.0 cm.
48 cm.	17 min.	2.82 cm.	145.2 cm.
52 cm.	20 min.	2.60 cm.	156.0 cm.
41 cm.	15 min.	2.73 cm.	163.8 cm.
36 cm.	13 min.	2.76 cm.	165.6 cm.
29 cm.	10 min.	2.90 cm.	174.0 cm.
60 cm.	15 min.	4.00 cm.	240.0 cm.
47 cm.	12 min.	3.91 cm.	234.6 cm.
35 cm.	10 min.	3.50 cm.	210.0 cm.

In obtaining the data tabulated in table III, I have employed SACHS's lithium method, and find that the rate of sap-flow in potted *Cyperus* plants and of plants in nutrient solutions varies from a minimum of 145 cm. per hour to a maximum of 240 cm. per hour. In cut plants the flow is slightly more rapid, the extremes being 180 and 250 cm. per hour. In comparison with *Helianthus*,

therefore, the rate of sap-flow in *Cyperus* is exceedingly rapid. Certain other herbaceous plants, however, have been reported as having a still more rapid flow, STRASBURGER observing that the rate of rise was 6 m. per hour in *Bryonia* and *Cucurbita*. We see, therefore, that *Cyperus* may at least be regarded as a rapidly transpiring plant, that its transpiration stream is correspondingly rapid, and that the amount of water given off from plants of relatively the same age and development show very little variation.

Experiments with heat

Over 200 experiments in which portions of the stems were killed with steam have been performed and the results carefully compared. The results of all of them are substantially in accord. The results obtained in several representative experiments are tabulated in table IV.

In table IV the numbers in the first column on the left refer to the actual numbers of the experiments performed; for example, 6—S (1908) means the sixth experiment in 1908 in which a portion of the stem (20 cm. in this case) was killed with steam. Each year the experiments performed were numbered anew. In the second column the length of the stem from the soil to the involucre is tabulated. The length of the steamed portion varies from a minimum of 5 cm. to a maximum of 30 cm., as will be seen from the third column; the time of treatment was 10–30 minutes. In the fourth column the actual day and month when the steam was applied are shown. The plants were in all cases grown in pots in soil under favorable greenhouse conditions. The control plants were stems cut from the same pot as the one which had been treated, and were approximately of the same size and age. They were set in water in the neighborhood, and the time of wilting of the leaves compared with those of a killed plant. As is well known, the leaves on cut stems of this plant not placed in water wither within 24–48 hours. I have omitted from this table the distance above the base of the stem at which the steam was applied, since I have found that it makes no appreciable difference whether it is applied near the leaves or lower down.

It is plain that the leaves of umbrella plants which have a portion

TABLE IV

SHOWING RESULTS OF KILLING 5 TO 30 CM. OF THE STEM WITH STEAM, AS
COMPARED WITH UNTREATED PLANTS CUT AND SET IN WATER

No. of exp.	Height of stem	Length of steamed portion	Time of treatment	Date	Results and comparisons
6—S (1908)	30 cm.	20 cm.	15 min.	Jan. 20	Leaves remained perfectly fresh and turgid for 7 days; Jan. 28 began to droop; withered Jan. 30, or in 10 days from time of treatment; control plant, cut and set in water, withered in 8 days.
11—S (1908)	40 cm.	15 cm.	15 min.	Feb. 2	Fresh and turgid for 8 days; Feb. 11, showed signs of withering; entirely wilted Feb. 13, or after 11 days; control plant in water withered in 6 days.
14—S (1908)	20 cm.	10 cm.	15 min.	Mar. 5	Fresh and turgid after 8 days; March 13, began to wither; entirely withered March 18, after 13 days; control in water withered in 8 days.
50—S (1908)	15 cm.	10 cm.	15 min.	June 15	Perfectly turgid after 8 days; withered June 24; dry June 26; control plant in water dry in 8 days.
5—S (1909)	20 cm.	10 cm.	15 min.	Mar. 15	Results exactly the same as in exp. no. 11—S (1908), except control in water was entirely dry in 7 days.
24—S (1909)	35 cm.	10 cm.	20 min.	April 5	Turgid for 8 days; withering April 15; entirely withered April 16, or after 10-11 days; control in water dry in 9 days.
31—S (1909)	25 cm.	15 cm.	20 min.	April 11	Withered on 11th day, April 22; control in water turgid for 9 days.
48—S (1909)	45 cm.	15 cm.	20 min.	April 19	Fresh and turgid for 10 days; withered April 30; control in water turgid for 9 days.
56—S (1909)	40 cm.	20 cm.	30 min.	April 22	Remained fresh for 4 days; April 27, showed signs of withering; next 2 days rapid withering; April 30, quite dry; stem turgid above and below steamed part, but collapsed in tube where steamed; no controls.
62—S (1909)	52 cm.	30 cm.	15 min.	May 3	Showed decided withering in 3 days; entirely withered in 5 days and dry in 7 days; control in water turgid for 7 days and dry in 9 days.

TABLE IV—*Continued*

No. of exp.	Height of stem	Length of steamed portion	Time of treatment	Date	Results and comparisons
20—S (1910)	43 cm.	14 cm.	15 min.	Feb. 13	Remained turgid for 7 days, then leaves drooped, edges inrolled, and plant dried in 10 days; control in water withered in 6 days.
27—S (1910)	51 cm.	17 cm.	15 min.	Feb. 19	Remained turgid for 8 days; several leaves drooped on 9th day; dry in 7 days; control in water turgid for 7 days.
41—S (1910)	32 cm.	20 cm.	15 min.	Mar. 4	Turgid for 7 days; leaves drooping on 8th day, dry on 9th day; control in water withered in 7 days.
52—S (1910)	41 cm.	11 cm.	15 min.	Mar. 22	Turgid for 8 days, drying on 10th day, withered on 11th day, dry on 13th day; control in water withered in 8 days.
63—S (1910)	60 cm.	10 cm.	15 min.	Mar. 22	Fresh and turgid for 8 days, drooped on 9th day, and dry in 11 days; control in water withered in 8 days.
74—S (1910)	32 cm.	20 cm.	20 min.	April 16	Withered in 7 days, entirely dry in 8 days; control in water withered in 7 days.
101—S (1910)	58 cm.	25 cm.	22 min.	May 2	Wilted in 4 days, dry in 6 days; control in water withered in 7 days.
109—S (1910)	61 cm.	20 cm.	25 min.	May 11	Leaves drooping in 7 days, withered in 8 days, dry in 9 days; control in water; withered in 6 days, dry in 7 days.
121—S (1910)	32 cm.	8 cm.	15 min.	May 23	Drooped in 10 days, withering in 11 days, dry in 12 days; control in water withered in 6 days.
133—S (1910)	42 cm.	5 cm.	20 min.	June 7	Leaves turgid and fresh after 10 days except tips very slightly drying, withering in 16 days, dry in 18 days; control in water withered in 8 days.
137—S (1910)	52 cm.	28 cm.	10 min.	June 15	Withering on 5th day and drying on 6th day; control in water withered in 7 days.

of their stems killed with steam remain turgid for a considerable time, and do not wither quite so quickly as those on stems cut and set in water. On treated stems the leaves remain turgid 5-18 days, while on stems cut and set in water they remain turgid

for a period not to exceed 9 days. The longer the steamed portion of the stem is, the sooner the leaves above lose their turgidity, wither, and dry. This is conspicuous in the cases of the plants in experiments 6—S (1908), 56—S (1909), 62—S (1909), 27—S (1910), 74—S (1910), 101—S (1910), 109—S (1910), and 137—S (1910), as compared with the others in which shorter sections were killed, as in 14—S (1908), 50—S (1908), 5—S (1909), 24—S (1909), 63—S (1910), and especially 133—S (1910) and 137—S (1910).

When plants of *Cyperus* have a portion of the stems steamed, the leaves above show no immediate effect. After some time, depending upon the length of the stretch heated, the involucre rays usually begin to droop, closing more or less about the stem. Loss of turgidity then follows, the margins of the rays become rolled inward, and dryness supervenes. In some cases discoloration follows the treatment, the leaves becoming spotted. This discoloration usually takes place at the tips first, but may occur also at the bases of the rays. I have noticed that this discoloration is dependent upon the length of the stem killed. When very short portions are steamed, the leaves usually wither and dry without discoloring, behaving like those on cut stems in this respect. When, on the other hand, longer portions are heated, discoloration usually follows. This fact seems to me to indicate, as DIXON maintains, that poisonous substances may be carried to the leaves from the killed portion.

In comparing the above results with those of experiments on Gramineae as described by ROSHARDT, in which 10 to 20 cm. of the halms were steamed, *Cyperus* appears to be able to retain the turgidity of its leaves after such treatment about as long as any of the grasses. The leaves finally droop at the bases, so that the whole involucre closes about the stem like the ribs of a closed umbrella. This is always the first sign that the plant has suffered from the treatment; yellowing of the tips and bases may also occur; and finally the leaves dry out after, in some cases, assuming a drooping position. In some instances the edges of the drying leaf become inrolled. Perhaps the initial drooping of the leaves or loss of turgidity may be due to some toxic action initiated by the treatment. Leaves on cut stems of *Cyperus* lose their turgidity and

droop in 1-2 hours, and very soon become dry, agreeing in this respect with the behavior of the leaves of *Bromus hordeaceus*. My observations support ROSHARDT's conclusions on this point.

By the method described above, plants growing in nutrient solutions may be conveniently sealed in the culture bottles without harming the plants, and the amount of transpiration from day to day may easily be determined by weighing. In order to ascertain whether less water is carried to the leaves after steaming, the rate of transpiration during each 24 hours for 5 days was determined, changes of temperature and the weather conditions being noted; then 10 cm. of the stem were killed with steam and the observations on rate of transpiration were continued. I have tabulated (tables V and VI) the results of such a series of observations.

TABLES V AND VI

SHOWING THE EFFECT ON AMOUNT OF WATER LOST DAILY BEFORE AND AFTER STEAMING 10 CM. OF STEM

TABLE V—OBSERVATIONS BEFORE STEAMING				TABLE VI—OBSERVATIONS AFTER STEAMING				
Date 1910	Loss in grams	Average tem- perature	Weather conditions	Date 1910	Loss in grams	Average tem- perature	Weather conditions	Remarks
May 18	4.0	24° C.	Clear	May 23	1.7	18° C.	Cloudy	Plant in good condition.
May 19	4.7	24° C.	Clear	May 24	1.5	25° C.	Cloudy	Stem above killed part withering.
May 20	5.8	25° C.	Clear	May 25	0.9	19° C.	Cloudy	Leaf tips very slightly drying.
May 21	3.2	39° C.	Cloudy, warm	May 26	0.7	20° C.	Clear	Leaves withered, dry, and in-rolled.
May 22	2.9	18° C.	Very cloudy	May 27	0.4	23° C.	Clear	Leaves entirely withered and dry.
Average transpiration in 24 hours=3.9 grams.				Average transpiration in 24 hours=1.04 grams.				

In tables V and VI the date of the observations is indicated in the first column, May 18-22, 1910 (before steaming 10 cm.), and May 23-27, 1910 (after steaming). In the second column the loss in grams before and after treatment is shown. There is a gradual decrease in the transpiration loss from 4 gm. to 2.9 gm. per day in table V, due probably to gradual lowering of the temper-

ature. The temperature was obtained, as previously, by taking the average of the temperatures recorded by the self-registering thermometer. After steaming, the diminishing transpiration cannot be due to decrease in temperature, since the temperature on the first day after killing the stem was the same as on the last day before steaming 10 cm. In table VI the average daily temperature gradually rises, while the transpiration diminishes from day to day, until on the last day (May 27) it is only 0.4 gm., having fallen off from 1.7 gm. on May 23. The weather conditions are recorded in order to show that decrease in transpiration is more dependent on the stem having been steamed than upon the changes of temperature and light.

It is evident from these tables that the amount of water transpired after steaming 10 cm. of the stem of *Cyperus* is very much less than before the treatment. The difference in temperature and weather conditions must be taken into account, but they are probably not sufficient to account for the very great difference in the amount of water given off in the two cases. In table V the average temperature record is higher than in table VI, and there was more clear weather, but these differences do not account for the immediate and continued slowing down of the transpiration rate as shown in table VI. The amount (1.7 gm.) transpired during the first 24 hours after steaming is far below that of any corresponding period before treatment. On a very cloudy day, at a temperature of 18° C., the plant gave off 2.9 gm. of water; while on a very bright day, at a temperature of 25° C., it transpired only 1.5 gm. after a section of 10 cm. had been steamed. It is plain that steaming 10 cm. of a stem of this plant very soon affects in some way the amount of water given off by the leaves.

In fairly mature shoots of *Cyperus*, the amount of water given off by the leaves in a given time may be assumed to be approximately equal to the amount absorbed by the roots. Therefore, the steaming must in some way interfere with the amount of water that passes through the killed portion, as well as that given off by the leaves. There is of course an almost immediate withering and shrinking of the steamed portions of the stem; if this region is exposed it soon dries out also. Careful weighing shows that

fresh turgid leaves of this plant contain about 80 per cent of their dry weight of water, while drying leaves on steamed stems directly after treatment, that is, 2-3 days, contain only about 50 per cent. As further noted in the table, the drying and withering proceed gradually, the leaves first drooping at their bases. There can be no doubt that steaming a section of stem reduces the water supply to the leaves, and that they thus come to contain less water. Whether this is the cause of withering, however, is not altogether clear. It may well be that there is water enough to maintain turgidity, even though the total amount has been reduced.

DIXON (9-12) concludes from experimental and histological evidence that the initial stages of fading are caused by a poisoning of the mesophyll cells of the leaves, while the final stages are accelerated by a clogging of the walls and a stoppage of the lumina of the conducting tubes. Upon microscopical examination of the steamed region, no visible disorganization of the cells except in the peripheral parenchyma could be found. In these cells the protoplasts were collapsed and the chloroplasts were discolored. The vascular bundles and internal parenchyma appear normal. When such stems are cut off and set in eosin, however, the color does not appear alone in the bundles, but diffuses out into the surrounding tissue. Since in these experiments the stem has been confined in a sterile chamber, there is no chance for the action of bacteria, and I have failed to find bacteria present in the tissues after a period of 10 days. Although no visible disorganization in this region can be made out with the microscope, it does not follow that disorganization has not occurred, and that certain decomposition products may not be exuded into the transpiration stream and be carried to other parts of the plant.

When steamed stems are split lengthwise, even without the aid of the microscope numerous dark streaks or lines in the tissues may be seen reaching as much as 15-20 cm. above the killed portion and often as far as the leaves. When the leaves are held up to the light, dark streaks can also be seen along some of the veins. I have studied both cross and longitudinal sections of the stems both above and below the steamed region. Under the microscope these streaks are seen to be vascular bundles, which

have been colored yellow or brown; the whole bundle is colored a yellowish brown or black. Nearly every bundle for some distance above the killed portion presents this appearance, while only occasionally one or more are seen colored below the region. The phloem is the part most deeply colored, being very dark brown and often almost black. A disorganization of the substances in the sieve tubes has apparently been caused by the heat; these substances can be seen to have penetrated the cell walls and to have passed out into the other elements of the bundle and also into the parenchyma cells. Under the microscope the substance which plugs the vessels appears like the mucilage or gum-resin so often present in liverwort gametophytes. It is yellowish in color and gelatinous in consistency; on testing with alkanin it is found to give the characteristic red reaction of resin. The xylem vessels also appear to be plugged with this same resinous substance. The smaller pitted vessels as well as the larger ones show this plugging, also the spiral and annular vessels, and even the large air spaces. It varies in degree; sometimes all of the xylem will be stopped, and then again only one or more vessels. The walls of the vessels also show the presence of this substance, and it is even present for some distance out in the surrounding parenchyma cells. When unstained with alkanin it is yellow or brown, depending upon the amount present; the deepest color is in the phloem, where it is nearly black, which goes to show that the substance originates here and diffuses out into the surrounding tissues. That it is not present in the killed portion itself is probably due to the fact that the heat vaporizes or expands the liquids and forces them upward and downward in the sieve tubes, and that the lateral diffusion into the surrounding tissues occurs later. This substance becomes visible very soon after steaming the stem.

From the above observations there can be no doubt that in this plant substances due to steaming are exuded into the water passages, which may possibly obstruct the upward flow of water and prevent an adequate amount reaching the transpiring leaves above. These facts alone may possibly be sufficient to account for the diminished transpiration and diminished water content of the leaves indicated in tables V and VI. Whether this clogging accounts wholly for

the withering and final drying of the leaves, as has already been suggested by DIXON, is a difficult question. Although URSPRUNG and ROSHARDT were unable to observe such clogging substances in all of the plants experimented upon by them, they are entirely too conspicuous to be disregarded in *Cyperus*. DIXON (10) has also found clogging substances in *Populus* stems which have been treated with steam or hot water.

I have also made experiments using decoctions of the stems of these plants as a water supply. After thorough sterilization of the decoction, cut stems were set in it. In a very short time the xylem becomes plugged for some distance with the same resinous substance, judging from its reactions to alkanin, as stops the passages when steam is applied to a normal plant, while the phloem remains unstained. The leaves above soon lose their turgescence and rapidly fall off in the amount of water transpired.

When fresh stems are set in water, the xylem vessels finally become plugged with dark-colored material, which is probably a decomposition product, due to decay or bacterial action. In order to be sure that the plugging of the stems set in the decoction was not due to bacteria, as URSPRUNG believes to be the case in DIXON's experiments, I have examined both the solutions and the stems for the presence of bacteria and always with negative results.

To further test DIXON's hypothesis that heating a section of the stems causes substances to be formed which poison the plant, I have performed a series of experiments as follows. Nutrient solutions were made, in which a sterilized plant decoction instead of distilled water was used and the requisite salts added thereto. Plants grown in such nutrient solutions very soon show signs of fading and the leaves become discolored, behaving in all respects much like those with steamed stems. A plant was sealed in such a culture solution on June 3 and kept under the most favorable conditions; the bottle was sealed to keep the solution sterile. In 3 days thereafter the leaves began to droop, and in 5 days began to show signs of discoloration along the veins; in 7 days the leaves were streaked with yellow and appeared like those on steamed stems. A control plant, which was set up in an ordinary nutrient solution in the same way at the same time, remained perfectly normal. The con-

ditions were identical with the exception of the use of the decoction in making up the nutrient solution.

Sections were made of the leaves from a stem killed as just described. The protoplasts of the mesophyll cells were much contracted and the chloroplasts were discolored. The same thing is true of the leaves on a stem which has stood for some time in a sterilized stem decoction. The leaves from plants grown in a stem decoction also show similar appearances. All of my observations go to show that the plant after steaming is poisoned. DIXON (10) finds that discoloration of the leaves above the killed portion, and even on a separate branch if its water supply must pass through such a heated region, may occur. This he thinks is due to a poison, since leaves which wither simply from lack of water shrivel while they are still green. In *Cyperus* I have observed no such marked discoloration of leaves before shriveling. In many cases the leaves on a steamed stem dry without any discoloration whatever, although the protoplasts of the mesophyll may be completely plasmolyzed long before shriveling occurs. In other cases the leaves become discolored.

URSPRUNG believes that experiments with cut branches are untrustworthy in such problems as the one under consideration, since the conditions are not the same as when the plant is in connection with the roots. I have made a series of experiments to compare the results with cut branches with those obtained from rooted plants. The average daily rate of water loss was obtained for each stem, and then a portion of the stem killed with steam or hot wax and the weighings continued. That these cut stems behave like those in connection with the roots the following results will show. A section 10 cm. long of one of two similar cut stems, each of which gave off approximately 7 gm. of water in 24 hours, was steamed for 30 minutes; during the next 24 hours this amount decreased to 3.2 gm., and to 0.5 gm. in the third period, and the leaves were very dry. A control plant transpired 5.2 gm. during the first 24 hours, 4.7 gm. during the second 24 hours, and 4.2 gm. during the third period. We see that in cut plants there is the same immediate and continued falling off in transpiration after steaming the stem as occurs when the stem is similarly treated

but left connected with the roots. A section of the stem 10 cm. long of one of two similar cut plants whose daily water loss was 5.4 gm. was immersed for 20 minutes in hot wax, which was allowed to cool about the stem. This plant gave off 5.6 gm. of water during the next period of 24 hours, an actual increase over the amount given off before treatment; the control gave off 5 gm. during the same period. During the third period the plant with the dead section of stem gave off 1.8 gm. of water, and the control 2 gm. It seems evident from these results that steam has the same effect on the transpiration of cut stems set in water that it does on normal plants.

Judging from the behavior of the leaves on a stem with a steamed section, from the collapse of the mesophyll cells, and from the apparent disorganization induced by steaming as indicated by the plugging of the water passages, I have been led to conclude that this method of killing the cells is not a satisfactory one in order to settle the question as to the relation of living cells to sap-flow. I agree with CZAPEK that the killing of the cells should be accomplished without further disorganization of the transpiring tissues. I have tried, therefore, to find a more satisfactory method of killing the cells of the stem without causing so much disturbance and disorganization as is induced by steam. I have surrounded the stem in the region to be killed with hot paraffin or wax, by pouring it into the incasing glass tube at a temperature of 110° C. and allowing it to cool and set about the stem, thus killing it and protecting it during the experiment. In some cases I have found it necessary to protect the stem further with grafting wax. On microscopical examination I find that the portions thus treated are quite dead, the parenchyma cells having their protoplasts contracted, and the peripheral mesophyll cells being discolored, with their protoplasts contracted. No change could be observed in vascular bundles. In such stems when cut and set in eosin, the color passes up mainly in the tracheae, and does not diffuse laterally into the surrounding tissue as in steamed stems. I also find that much less plugging of the vessels above the killed portion occurs after this method, as well as less plasmolysis of the mesophyll cells. In every respect I think this method of applying heat is far superior to steaming,

though some plugging of the lumina occurs. I have tabulated (table VII) the results obtained by this method for comparison with those obtained in experiments with steam.

TABLE VII

SHOWING RESULTS OF KILLING CERTAIN PORTIONS OF THE STEMS WITH WAX HEATED TO 110° C. AS COMPARED WITH UNHEATED STEMS CUT AND SET IN WATER

No. of exp.	Height of stem	Length of killed portion	Date	Results and remarks
1—W (1910)	65 cm.	6 cm.	May 7	Leaves began to wither May 23, after 16 days; control in water dry in 5 days; wax relaxed about stem, exposing it to drying.
2—W (1910)	60 cm.	12 cm.	May 7	May 23, leaves slightly drooped but perfectly turgid; May 25, leaves quite drooping, tips drying and inrolling; May 26, leaves drying and inrolled; dry after 19 days, cut off for examination; control same as in no. 1—W.
3—W (1910)	50 cm.	10 cm.	May 19	June 11, leaves drooping; tips slightly inrolled; withering June 16; involucre dry after 23 days; control in water dried after 7 days.
4—W (1910)	53 cm.	10 cm.	May 19	Leaf tips drying and inrolling, slightly yellow; wax relaxed about stem; dry after 14 days; cut off for examination; portion above killed region showed yellow streaks about bundles, but no plugging of the lumina; control same as in no. 3—W.
5—W (1910)	50 cm.	40 cm.	May 19	May 20, some leaf tips drooping; May 23, leaves beginning to dry; May 25, plant dry, after 5 days only; cut off stem and set in eosin, color rose in vessels only; no visible plugging of lumina; control same as in no. 3—W.
6—W (1910)	30 cm.	20 cm.	May 22	June 2, leaves beginning to dry; June 3, leaves quite dry; June 5, leaves entirely dry; control in water dry in 7 days.
7—W (1910)	40 cm.	10 cm.	May 22	June 6, involucre still turgid, but 3 rays beginning to turn yellow, other leaves perfect in color; June 18, all leaves yellowing; June 20, plant quite dry; control dry in 8 days.
8—W (1910)	34 cm.	5 cm.	May 22	June 9, plant still in perfect condition except the portion killed; all of the leaves green and turgid and none show the slightest signs of injury or discoloration; at this writing (48 days after treatment) leaves show no signs of wilting; control dry in 8 days.

Experiments in which steam was employed to kill certain stretches of the stem, as indicated in table IV, show that the leaves

above such steamed regions remain turgescient for a period of 5-18 days, depending on the length of the stretch killed. Comparing the results of table VII with those of table IV, we see that in killing by hot wax, the longer the killed section, the sooner the leaves above wither. On the other hand, this method of applying heat does not cause withering and final drying nearly so quickly as steaming. There can be no doubt that the treated portions were killed by the hot wax. Microscopical examination indicates that the cells are dead, but that there is much less disorganization caused by this sort of treatment than by using steam. These stems do not show so much initial plugging of the lumina of the vessels, although the ducts finally become much discolored. As the table shows, leaves may remain turgid for a period of 5-23 days (or longer in case 5 cm. are killed), depending on the length of the stretch killed; while the maximum time of turgidity for stems with a section killed by steam did not exceed 18 days. To compare specific cases in table IV, nos. 14—S (1908), 50—S (1908), 5—S (1909), 24—S (1909), and 63—S (1910) each had 10 cm. treated; the leaves on these plants withered in 8 or 10 days. In table VII, nos. 3—W, 4—W, and 7—W each had the same distance killed, but the leaves did not dry until 14-34 days thereafter. They lasted nearly three times as long.

I have also determined the actual amount of water transpired by the leaves of a plant before and after treatment with the hot wax, just as was done in the case when steam was used (see tables V and VI). The average transpiration for each 24 hours for 5 days was determined, then 10 cm. of the stem were killed and the observation was continued. The results appear in tables VIII and IX. The daily loss in grams is shown in the second column in each table. Observations were made in order to compare the effect of temperature and weather conditions on the amount of transpiration. The average daily temperature was obtained, as before, by taking the average of the temperatures as recorded by the self-registering thermometer.

It will be noted that there is an increased amount of water transpired the first 2 days after treatment with the hot wax, although the temperature was lower than it was the first 2 days

before such treatment; there is also an increase on the fifth day over the fourth. In the case of stems with steamed sections no such increase occurs, as is shown by table VI. The steaming causes an immediate and continued falling off in the amount of water transpired. The plants used for the experiment, the results of which are tabulated in tables VIII and IX, were examined microscopically and tested with alkanin, and very little plugging of the lumina was found. No such discoloration of the parenchyma surrounding the bundles was observed as occurs in steamed stems.

TABLES VIII AND IX

SHOWING THE EFFECT ON DAILY TRANSPIRATION BEFORE AND AFTER KILLING 20 CM. OF THE STEM WITH WAX HEATED TO 110° C.

TABLE VIII—OBSERVATIONS BEFORE KILLING				TABLE IX—OBSERVATIONS AFTER KILLING				
Date 1910	Loss in grams	Average tem- perature	Weather conditions	Date 1910	Loss in grams	Average tem- perature	Weather conditions	Remarks
May 17	1.1	19° C.	Cloudy	May 22	1.9	19° C.	Clear	Plant in good condition
May 18	2.0	24° C.	Clear	May 23	2.6	20° C.	Cloudy	Plant in good condition
May 19	2.5	24° C.	Clear	May 24	1.8	21° C.	Cloudy	Plant in good condition
May 20	2.7	25° C.	Clear	May 25	1.4	26° C.	Cloudy	Plant in good condition
May 21	2.3	29° C.	Slightly cloudy	May 26	1.6	26° C.	Slightly cloudy	Leaf tips very slightly yellow
Average loss in 24 hours = 2.12 grams.				Average loss in 24 hours = 1.86 grams.				

The leaves also showed very little plasmolysis in the mesophyll cells, and the chloroplasts were in good condition. These results undoubtedly indicate that hot wax is superior to steam for killing the cells of stems without disarranging the tissues.

In view of SCHROEDER's observations, it will be instructive to examine again the results shown in table VI. After 10 cm. of the stem were steamed, a successive diminution in the amount of water lost in each 24 hours occurs. The leaves behave exactly as though they were wilting from lack of water supply, except that the process is not so rapid, owing probably to a certain amount of water being able to rise in the not completely plugged vessels. It is further

to be noted that after the second day the amount falls from 1.5 gm. to 0.9 gm., after which there is a more gradual daily diminution in the amount transpired. This seems to indicate, in the light of SCHROEDER'S results, that the leaves were dying, which is undoubtedly the case. Death is probably due to deleterious substances being introduced into the leaves from the killed portion of the stem. Examining table IX, in which the results are shown after using hot wax as a killing medium, we see that on the second day the plant increases the amount of water given off, which proves that it is not dying. There is also an increase on the fifth day over the fourth. I suspect that the leaves in this case have not been so severely poisoned as in the plant killed with steam.

That withering of the leaves on stems, which have been killed with heat, is not due to lack of water, but to the toxic action of substances which have been carried to the leaves, seems to be shown by the above-described experiments. The fact that the longer the heated region, the more rapid the leaves wither, seems to favor such a view. That leaves above a steamed or otherwise heated portion do not wither in the same way as those simply deprived of water, but often discolor before shriveling, also supports such a conclusion, as DIXON has already shown.

Histological examination of such leaves shows that the protoplasts and chloroplasts resemble those under diseased conditions more than those in leaves which are merely drying for lack of water supply. I agree with DIXON that leaves on cut branches die from lack of water supply, while in the case of leaves borne above a heated portion of the stem, they die because they dry.